



2,254-101 A.

Case Docket No. P564-7015
Date June 24, 1997

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of
Inventor(s): Dolores J. SCHENDEL

For: T CELLS SPECIFIC FOR KIDNEY CARCINOMA

- ☒ Specification and Claims (44 pages)
- ☒ 6 sheets of drawings
- ☒ Declaration and Power of Attorney
- ☒ An assignment of the invention to Boehringer Mannheim GmbH with accompanying PTO-1595 Form
- ☒ A certified copy of German application No. 196 25 191.5 filed: June 24, 1996
- ☒ A filing fee, calculated as shown below:

	(Col. 1)	(Col. 2)
FOR:	No. Filed	No. Extra
BASIC FEE		
TOTAL CLAIMS	72 - 20 =	* 52
INDEP CLAIMS	4 - 3 =	* 1
<input checked="" type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENTED		

* If the difference in Col. 1 is less than zero, enter "0" in Col. 2

Small Entity	
RATE	FEE
	\$385
x 11 =	0
x 40 =	0
+130 =	0
TOTAL	0

Other Than A Small Entity	
RATE	FEE
	\$770
x 22 =	1144
x 80 =	80
+260 =	260
	2254

or
or
or
or
or
or

☒ A check in the amount of \$2,294 to cover the filing fee and \$40 assignment recordation fee.

☒ In the event that the attached check is found to be insufficient, the Commissioner is hereby authorized to charge payment for any additional filing fees required under 37 CFR 1.16 associated with this communication or credit any over-payment to Deposit Account No. 14-1060.

Respectfully submitted,

NIKAIDO, MARMELSTEIN, MURRAY & ORAM LLP

By: Robert B. Murray
Reg. No. 22,980

Metropolitan Square
655 15th Street, N. W.
Suite 330 - G Street Lobby
Washington, D. C. 20005-5701
Tel: (202) 638-5000
Fax: (202) 638-4810
RBM/cb
OK #13766

Boehringer Mannheim GmbH
Sandhofer Str. 112-132

68305 Mannheim-Waldhof

T cells specific for kidney carcinoma

T cells specific for kidney carcinoma

Description

The present invention concerns new nucleic acid and amino acid sequences of the human T cell receptor and their use for the diagnosis and therapy of carcinomas in particular of kidney cell carcinomas.

The T lymphocytes of the immune system are responsible for the cellular immune response. They are able to recognize and eliminate diseased body cells, e.g. cells which contain foreign proteins, or tumour cells. Diseased body cells are recognized by the so-called T cell receptor (TCR) which binds an antigen in the form of short peptide fragments which is specific for the diseased cell. These peptide fragments are presented by MHC molecules on the cell surface.

T cell receptors are composed of two different polypeptide subunits, usually the so-called T cell receptor α or β chains which are linked together by a disulfide bridge. The α and β chains are in turn composed of variable and constant regions. The variable regions of the α chain comprise V and J gene segments and the variable regions of the β chain comprise V, D and J gene segments.

The TCR α chain gene is composed of over 100 variable segments each of which contains an exon for a V region in front of which there is another exon which codes for a leader sequence which enables transport of the protein to the cell surface. A group of 61 J segments lies at a

considerable distance from the V segments. The J segments are followed by a single C segment for the constant region which in turn contains separate exons for the constant region and the hinge region as well as an exon for the transmembrane and cytoplasm regions.

The TCR β chain gene contains a group of approximately 30 V gene segments which are at some distance from 2 separate clusters which each contain a single D segment and 6 or 7 J segments as well as a single C segment. Each constant segment of the β chain has separate exons for the constant, the hinge, the transmembrane and the cytoplasm region.

During the development of the T cell the separate segments are linked by somatic recombination. In the case of the α chain a $V\alpha$ gene segment gets next to a $J\alpha$ gene segment and hence a functional exon is formed. Transcription and splicing of the $VJ\alpha$ exon to the constant region leads to the formation of the mRNA which is translated into the TCR α chain. The rearrangement of the $V\beta$, $D\beta$ and $J\beta$ gene segments coding for the variable domain of the β chain creates a functional exon which is transcribed and attached to $C\beta$ by splicing. The mRNA which forms is translated into the TCR β chain. The α and β chains join together after their biosynthesis to form an $\alpha : \beta$ TCR heterodimer. The highly variable region of the TCR which is responsible for the specificity of antigen recognition and is located in the linkage region of the V, (D) and J gene segments is referred to as the CDR3 region.

Due to the high variability of T cell receptors it is very time-consuming to identify specific nucleotide and

amino acid sequences in particular in the area of the CDR3 antigen recognition region. There is therefore a great need to provide nucleic acid and amino acid sequences of T cell receptors which are able to specifically recognize clinically relevant peptide antigens in particular tumour-specific peptide antigens.

According to the invention tumour-infiltrating lymphocytes (TIL) could be isolated from a kidney carcinoma which have a high specificity for tumour tissue from patients with the HLA-A*0201 allele. These TIL show no reaction with healthy kidney tissue from the same patient.

An analysis was carried out of the nucleotide and amino acid sequences of the T cell receptors expressed by these TIL. In this process a homogeneous CD8⁺ T cell clone was firstly obtained by culturing and periodic restimulating the TIL over a period of 62 and 74 days respectively. The cDNA coding for the α and β chain of the T cell receptor was sequenced. The nucleotide and amino acid sequence of the α chain are shown in the sequence protocols SEQ ID NO. 1 and SEQ ID NO. 2. The CDR3 α region in SEQ ID NO. 1 extends from bp 313 to 348 corresponding to the amino acids 87-98 in SEQ ID NO. 2. The nucleotide and amino acid sequence of the β chain are shown in the sequence protocols SEQ ID NO. 3 and SEQ ID NO. 4. The CDR3 β region in SEQ ID NO. 3 extends from bp 331 to 369 in SEQ ID NO. 3 corresponding to the amino acids 90-102.

In the case of the α chain a combination of V α 20 with J α 22 was found in the variable region and in the case of the β chain a combination of V β 22, D β 2 and J β 2.7.

Subsequently a sequence analysis of the tumour-specific T cell receptors was carried out with a culture for only 24 days. In this case a homogeneous T cell clone was not found but rather a mixture of several T cell species. The amino acid sequence shown in SEQ ID NO. 2 as well as in all two further amino acid sequences were able to be identified for the α chain. 11 out of 56 examined T cell species coded for the amino acid sequence shown in SEQ ID NO. 2 of the CDR3 α region from position 87 to 98. the nucleotide sequence of the α chains in these T cells differed from the sequence shown in SEQ ID NO. 1 only by a substitution of T by G at position 324.

The nucleotide and amino acid sequence of the CDR3 region of a further α chain which was identified in 38 out of the 56 examined T cells is shown in the sequence protocols SEQ ID NO. 5 and 6. In addition two further T cell species were identified which contained a CDR3 α region with the same amino acid sequence to that shown in SEQ ID NO. 6 but whose nucleotide sequence each differed by a base substitution (C at position 9 substituted by G or T at position 12 substituted by C).

The nucleotide and amino acid sequence of the CDR3 α region from a third T cell variant which occurred at a frequency of 5 out of 56 examined T cell species is shown in the sequence protocols SEQ ID NO. 7 and 8.

The corresponding sequencing of the β chains yielded a total of 6 different amino acid sequences for the CDR3 region. A CDR3 β sequence which was found in 15 out of 50 examined T cells is shown in the sequence protocols SEQ ID NO. 9 and 10. A further T cell species contained the same amino acid sequence but a different nucleotide

sequence (substitution of A at position 15 by T).

One T cell species in each case contained the nucleotide and amino acid sequences shown in the sequence protocols SEQ ID NO. 11 and 12, 13 and 14 or 15 and 16 in the CDR3 β region.

27 out of 50 clones contained the nucleotide and amino acid sequences shown in the sequence protocols 17 and 18 in the CDR3 β region. 4 out of 50 examined clones contained the nucleotide and amino acid sequences shown in the sequence protocols SEQ ID NO. 19 and 20 in the CDR3 β region.

In addition an in situ sequencing of TIL was carried out i.e. a sequencing without prior culture. For this the entire RNA was isolated from the tumour, a TCR-specific cDNA was prepared using a TCR α - or TCR β -specific primer and reverse transcriptase and this cDNA was selectively amplified by PCR using family-specific primers (V α 20 and V β 22). The amplification products were cloned into E. coli and sequenced. In this process a series of single sequences was obtained.

Circa 60 % of all sequences of the α chain correspond to the amino acid sequences shown in the sequence protocols SEQ ID NO. 2, 6 and 8. A further 20 % had very similar sequences which were also composed of a combination of V α 20 and J α 22. An overview of the CDR3 α regions identified in this in situ sequencing of T cells from patient 26 is shown in Fig. 1.

Furthermore it was found in the in situ sequencing that ca. 70 % of all sequences of the β chain correspond to

the amino acid sequences shown in the sequence protocols 4, 10, 12, 14, 16, 18 and 20. An overview of the CDR3 sequences of the β chain identified in the in situ sequencing is shown in Fig. 2.

In a control experiment TIL from another patient with the HLA-A*0201 allele were analysed by in situ sequencing. It was found that the CDR3 α regions of 15 and 4 of the total of 34 examined T cell species contained the amino acid sequences shown in SEQ ID NO. 2 and SEQ ID NO. 6. An overview of the relevant CDR3 α sequences and their frequency is shown in Fig. 3. An overview of the results which were obtained when sequencing the CDR3 regions of the β chain is shown in Fig. 4.

Hence a first aspect of the present invention concerns a nucleic acid which codes for the α chain of a human T cell receptor, a functional derivative or a fragment thereof and comprises a CDR3 region composed of a combination of a V α 20 gene segment and a J α 22 gene segment. The length of the amino acid section coded by this CDR3 region is 11-14 amino acids and preferably 12 or 13 amino acids. The CDR3 region particularly preferably codes for one of the amino acid sequences shown in the sequence protocols SEQ ID NO. 2, 6 and 8, a sequence that is at least 80 % and in particular at least 90 % identical to this or a sequence which codes for an amino acid sequence with an equivalent recognition specificity for the peptide component of the T cell receptor ligand.

A further aspect of the present invention is a nucleic acid which codes for the α chain of a human T cell

receptor, or for a functional derivative or a fragment thereof and comprises a CDR3 region selected from:

- (a) a nucleotide sequence coding for the amino acid sequence



in which $X_1 \dots X_n$ represents a sequence of 3-5 amino acids,

- (b) a nucleotide sequence which codes for an amino acid sequence which is at least 80 % and in particular at least 90 % identical with the amino acid sequence from (a) or
- (c) a nucleotide sequence which codes for an amino acid sequence with an equivalent recognition specificity for the peptide component of the T cell receptor ligand.

The amino acid sequence $X_1 \dots X_n$ is preferably selected from the group comprising the amino acid sequences VGG, VLSG, ATG, VSG, DSG, VVSG, ALAG, APSG and VGR. The amino acid sequence $X_1 \dots X_n$ is particularly preferably selected from the amino acid sequences VGG, VLSG and ATG.

A particular feature of the tumour-specific CDR3 α regions of the invention is a length of 12-13 amino acids and a common sequence motif. Thus if the sequence $X_1 \dots X_n$ has a length of 3 amino acids X_1 is preferably V or A, X_2 is preferably T, G or S and X_3 is preferably G. If the length of the sequence $X_1 \dots X_n$ is 4 amino

acids then preferably $X_1 = V$ or A , at least one of X_2 or X_3 is T or S and $X_4 = G$.

A sequencing of the β chains from both patients that were examined yielded a combination of the gene segments $V\beta 22$, $D\beta 1$ or $D\beta 2$ and $J\beta 2.7$ for the first patient and a combination of the gene segments $V\beta 22$, $D\beta 1$ or $D\beta 2$ and $J\beta 2.1$, $J\beta 2.3$ or $J\beta 2.7$ for the second patient.

Hence a further aspect of the present invention is a nucleic acid which codes for the β chain of a human T cell receptor, or for a functional derivative or a fragment thereof and comprises a CDR3 region which is composed of a combination of a $V\beta 22$ gene segment of a $D\beta 1$ or $D\beta 2$ gene segment and of a $J\beta$ gene segment in particular of a $J\beta 2.1$, $J\beta 2.3$ or $J\beta 2.7$ gene segment.

The length of the amino acid section coded by this CDR3 β region is 12-14 amino acids, preferably 13 amino acids. Furthermore this CDR3 β region preferably contains a common sequence motif i.e. $X-T$ or $S-X-S$ in which X represents an arbitrary amino acid and T or S particularly preferably denote T . A total of 70 % of the examined T cell receptors have such a sequence pattern.

Yet a further aspect of the present invention is a nucleic acid which codes for the β chain of a human T cell receptor, or for a functional derivative or a fragment thereof and comprises a CDR3 region which is selected from:

- (a) a nucleotide sequence coding for the amino acid sequence

C A (X'₁ ... X'_n) Y/D E Q Y F (II)

in which X'₁ ... X'_n represents a sequence of 5-7 amino acids,

- (b) a nucleotide sequence coding for the amino acid sequence

C A (X''₁ ... X''_n) N E Q F F (III)

in which X''₁ ... X''_n represents a sequence of 5-7 amino acids,

- (c) a nucleotide sequence coding for the amino acid sequence

C A (X'''₁ ... X'''_n) D T Q Y F (IV)

in which X'''₁ ... X'''_n represents a sequence of 5-7 amino acids,

- (d) a nucleotide sequence which codes for an amino acid sequence that is at least 80 % and in particular at least 90 % identical with an amino acid sequence from (a), (b) or/and (c), or
- (e) a nucleotide sequence which codes for an amino acid sequence with an equivalent recognition specificity for the peptide component of the T cell receptor ligand.

The amino acid sequence X'₁ ... X'_n is preferably selected from the group comprising SSETNS, SSETSS,

TSGTAS, RSGTGS, SSGTDS, SSGTRS, SSGSDS, SSSTGS, SSSTVS, SSSTLS, SSSTLF, SSSTAS, SSHTDS, SSDTLS and SRWDSE. The amino acid sequence $X'_1 \dots X'_n$ particularly preferably represents SSETNS, SSGTDS, TSGTAS or RSGTGS. The amino acid sequence $X''_1 \dots X''_n$ preferably denotes SSGTSSY or SSDQGM. The amino acid sequence $X'''_1 \dots X'''_n$ preferably denotes SADSFK.

Within the sense of the present invention the term "functional derivative of a chain of a human T cell receptor" is understood as a polypeptide which comprises at least one CDR3 α or/and CDR3 β region as defined above and together with the respective complementary chain of the human T cell receptor (or a derivative of such a chain) can form a T cell receptor derivative which has an equivalent recognition specificity for a peptide ligand presented by a MHC molecule to that of the non-derivatized T cell receptor. Such a T cell receptor has a binding constant of at least 10^{-4} l/mol, preferably 10^{-4} to 10^{-5} l/mol for the presenting peptide ligand.

Functional derivatives of chains of a human T cell receptor can for example be prepared by deletion, substitution or/and insertion of sections of the gene coding for the respective polypeptide by means of recombinant DNA techniques. The preparation of recombinant T cell receptor chains is for example described in Blank et al. (1993), Eur. J. Immunol. 23, 3057-3065; Lin et al. (1990) Science 249: 677, Gregoire et al. (1991), Proc. Natl. Acad. Sci. USA, 88: 8077; Kappes and Tonegawa (1991), Proc. Natl. Acad. Sci. USA 88: 10619 and Ward (1991), Scand. J. Immunol. 34: 215. Explicit reference is herewith made to these literature citations.

Particular preferred functional derivatives of T cell receptor chains or T cell receptors are single chain T cell receptors which can for example be composed of the variable domains of the α and β chain and a constant domain. The preparation of such constructs is described by Chung et al. (1994), Proc. Natl. Acad. Sci. USA 91: 12654-12658. A further preferred example of functional derivatives are soluble TCR fragments which can be prepared as separate polypeptides or as single chain polypeptides cf. e.g. Hilyard et al. (1994), Proc. Natl. Acad. Sci. USA 91; 9057-9061. Explicit reference is also made to the disclosure in these literature citations.

A further subject matter of the present invention is a vector which contains at least one copy of a nucleic acid according to the invention. This vector can be a prokaryotic vector or a eukaryotic vector. Examples of prokaryotic vectors are plasmids, cosmids and bacteriophages. Such vectors are described in detail in Sambrook et al., Molecular Cloning. A Laboratory Manual, 2nd Edition (1989), Cold Spring Harbor Laboratory Press, in chapters 1-4. The prokaryotic vector is preferably a plasmid.

On the other hand the vector can also be a eukaryotic vector e.g. a yeast vector, a plant vector (baculovirus) or a mammalian vector (a plasmid vector or a viral vector). Examples of eukaryotic vectors are described in Sambrook et al., Supra, chapter 16 and Winnacker, Gene and Klone, "Eine Einführung in die Gentechnologie" (1985), VCH "Verlagsgesellschaft" in particular in chapters 5, 8 and 10.

Yet a further subject matter of the invention is a cell

which expresses a nucleic acid according to the invention or a cell which is transformed with a nucleic acid according to the invention or with a vector according to the invention. The cell can be a prokaryotic cell (e.g. a gram-negative bacterial cell, in particular *E. coli*) or a eukaryotic cell (e.g. a yeast, plant or mammalian cell). Examples of suitable cells and methods for introducing the nucleic acid according to the invention into such cells may be found in the above literature references.

A further subject matter of the present invention is a polypeptide which is coded by a nucleic acid according to the invention. The polypeptide particularly preferably contains the variable domain of the α or/and β chain of a human T cell receptor.

A polypeptide is particularly preferred which has T cell receptor properties and is composed of a TCR α chain or a functional derivative thereof as well as a TCR β chain or a functional derivative thereof as subunits. The polypeptide can be composed of two separate chains or be present as a single chain polypeptide. In addition the polypeptide may also be present in an oligomerized form in which at least 2 and preferably 2-10 TCR α and TCR β chains are linked together. The linkage can for example be achieved by means of bifunctional chemical linkers.

Yet a further subject matter of the present invention is an antibody against a polypeptide according to the invention which is directed towards a region of the polypeptide which is responsible for recognizing the peptide ligand. This antibody can be a polyclonal antiserum, a monoclonal antibody or a fragment of a

polyclonal or monoclonal antibody (e.g. a Fab, F(ab)₂, Fab' or F(ab')₂ fragment). The antibody is preferably directed towards a CDR3 region of the polypeptide or an area thereof. Such antibodies can be obtained by well-known methods by immunizing an experimental animal with a peptide or polypeptide which contains a CDR3 region according to the invention and isolating the resulting antibodies from the experimental animal. Monoclonal antibodies can be obtained by fusing an antibody-producing B cell of the experimental animal with a leukemia cell according to the method of Köhler and Milstein or further developments thereof. Specific examples of the production of such antibodies can be found in Choi et al. (1991), Proc. Natl. Acad. Sci. USA 88: 8357-8361 and Zumla et al. (1992), Hum. Immunol. 35: 141.

Yet a further subject matter of the present invention is a T cell which contains a T cell receptor according to the invention. Such T cells can be isolated from patients with kidney cell carcinoma and then be expanded in vitro. For this the peripheral mononuclear blood cells of a patient can for example be produced by stimulation with suitable antigens and subsequent restimulation for example with an irradiated autologous lymphoblastoid cell line, tumour cells, lymphoblastoid cells plus antigens or autologous peripheral blood lymphocytes plus antigen. Further methods for obtaining T cells according to the invention are described below.

The invention also concerns a pharmaceutical composition which contains a nucleic acid, a polypeptide, a peptide ligand capable of binding to the polypeptide optionally in association with a corresponding MHC molecule, an antibody or a cell as described above as active

components optionally together with other active components as well as common pharmaceutical auxiliary substances, additives or carrier substances. Examples of other active components are accessory stimulating components e.g. cytokines such as IL-2 and IL-4.

The pharmaceutical composition can be used to produce a diagnostic or therapeutic agent. Examples of diagnostic applications are the diagnosis of tumour diseases or a predisposition for tumour diseases. A further preferred diagnostic application is the monitoring of the disease course in a tumour disease e.g. after chemotherapy or a surgical operation.

The use of the pharmaceutical composition as a diagnostic agent preferably comprises the detection of a T cell subpopulation which expresses a polypeptide according to the invention as a T cell receptor. The detection of this T cell receptor can for example be achieved at the nucleic acid level e.g. by a nucleic acid hybridization assay optionally with a prior amplification. On the other hand the detection can also be carried out at the protein level by an immunoassay using antibodies that react specifically with the T cell receptor. In addition it is also possible to detect the T cells for example by means of a test for binding to specific peptide ligands or in an activity test in which the specific cytotoxic action of the T cells or the release of cytokines such as TNF or IFN γ is determined.

Furthermore the pharmaceutical composition according to the invention can also be used therapeutically in particular for the prevention or therapy of a tumour disease e.g. of a kidney cell carcinoma. This

therapeutic application can for example be based on the fact that T cells which express the tumour specific T cell receptor are stimulated to grow in vitro or in vivo. The growth stimulation in vivo can for example be achieved by administering the peptide ligand of the T cell receptor or/and the whole molecule from which the peptide ligand is derived or a fragment thereof. Furthermore the growth stimulation in vivo can also be accomplished by administering an antibody which specifically activates the T cell receptor by binding e.g. a monoclonal antibody or a monoclonal antibody fragment.

On the other hand the growth stimulation of the T cells can also be carried out in vitro for example by isolating specific T cells from the patient, in vitro expansion and subsequent administration of the expanded T cells as a tumour vaccine. T cells which express a tumour specific T cell receptor are isolated from a patient preferably by contacting a sample from the patient which contains T cells, e.g. a blood sample and preferably a sample derived from the tumour tissue, with an agent which specifically binds to the CDR3 region of the T cell receptor, identifying the T cells which react with the agent and optionally separating them from other T cells. The agent that binds to the CDR3 region of the T cell receptor is preferably selected from the peptide ligand of the T cells, a peptide ligand-MHC complex or/and an anti-TCR antibody. Optionally the in vitro expansion can additionally be carried out in the presence of costimulatory factors such as anti-CD28 antibodies. In order to facilitate separation of the desired T cell subpopulation, the agent is preferably used in an immobilized or immobilizable form.

The isolation of T cells which express a tumour specific T cell receptor can, however, also be achieved in another manner e.g. by introducing nucleic acid sequences which code for the T cell receptor into a T cell line, preferably a cytotoxic T cell line. The T cell receptor is then expressed in this transfected T cell line. In this manner it is possible to obtain T cells in large amounts which express a tumour specific T cell receptor.

Yet another method for isolating T cells which express a tumour specific T cell receptor is to introduce nucleic acid sequences which code for the T cell receptor into the germ line of an animal and to isolate the T cells from the resulting transgenic animal or its descendants. Transgenic mice are preferably produced. Furthermore it is preferred that the transgenic mice also express the human CD8 molecule or/and the human HLA-A*0201 molecule in addition to the T cell receptor.

Hence a further subject matter of the present invention is also a transgenic animal which has T cells that express a tumour specific T cell receptor. This transgenic animal is preferably a rodent in particular a mouse.

Finally the invention also concerns a method for the identification of peptide ligands of a T cell receptor according to the invention. This method preferably comprises the steps:

- (a) isolating RNA from tumour tissue,

- (b) converting the RNA into double-stranded cDNA molecules,
- (c) introducing the cDNA molecules into host cells to obtain a cDNA bank,
- (d) transfecting eukaryotic recipient cells with aliquots of the cDNA bank in which (i) there is a cotransfection with HLA-A*0201 DNA or (ii) HLA-A*0201-positive recipient cells are used,
- (e) testing the transfected recipient cells for their ability to stimulate T cells to for example proliferate or to secrete cytokines such as TNF in which case it is possible for example to examine the lysis of TNF-sensitive cells,
- (f) identifying a cDNA sequence which codes for the antigen which contains the peptide ligand and
- (g) identifying the sequence of the peptide ligand.

The invention is further elucidated by the following examples, figures and sequence protocols.

SEQ ID NO. 1: shows the nucleotide sequence of the TCR α chain of a T cell receptor according to the invention in which bp 55-324/325 codes for the TCR-V α 20 gene segment, bp 325/326 codes for the TCR J α 22 gene segment, bp 381-804 codes for the TCR-C α gene segment and bp 805-1341 represent a 3' untranslated region,

- SEQ ID NO. 2: shows the amino acid sequence of the
nucleotide sequence shown in SEQ ID NO. 1,
- SEQ ID NO. 3: shows the nucleotide sequence of the TCR β
chain of a T cell receptor according to
the invention in which bp 1-63 are
nucleotides, bp 346-349 code for the
TCR-D β 2 gene segment, bp 350 is an
N-nucleotide, bp 351-398 code for the
TCR-J β 2.7 gene segment and bp 399-936 code
for the TCR-C β gene segment,
- SEQ ID NO. 4: shows the amino acid sequence of the
nucleotide sequence shown in SEQ ID NO. 3,
- SEQ ID NO. 5
and 6 show nucleotide and amino acid sequences
of the CDR3 α region of a T cell receptor
according to the invention
- SEQ ID NO. 7
and 8: show nucleotide and amino acid sequences
of the CDR3 α region of a T cell receptor
according to the invention
- SEQ ID NO. 9
and 10: show nucleotide and amino acid sequences
of the CDR3 β region of a T cell receptor
according to the invention
- SEQ ID NO. 11
and 12: show nucleotide and amino acid sequences
of the CDR3 β region of a T cell receptor
according to the invention
- SEQ ID NO. 13
and 14: show nucleotide and amino acid sequences
of the CDR3 β region of a T cell receptor
according to the invention
- SEQ ID NO. 15
and 16: show nucleotide and amino acid sequences
of the CDR3 β region of a T cell receptor

according to the invention

SEQ ID NO. 17

and 18

show nucleotide and amino acid sequences of the CDR3 β region of a T cell receptor according to the invention

SEQ ID NO. 19

and 20:

show nucleotide and amino acid sequences of the CDR3 β region of a T cell receptor according to the invention

SEQ ID NO. 21 shows the nucleotide sequence of the TCR α -specific primer P-C α ST,

SEQ ID NO. 22 shows the nucleotide sequence of the TCR β -specific primer P-C β ST

Fig. 1

shows nucleotide and amino acid sequences of the CDR3 α regions from tumour-specific TCR which have been determined by in situ sequencing of T cells of patient 26,

Fig. 2

shows nucleotide and amino acid sequences of CDR3 β regions of tumour-specific TCR which have been determined by in situ sequencing of T cells of patient 26,

Fig. 3

shows nucleotide and amino acid sequences of CDR3 α regions of tumour-specific TCR which have been determined by in situ sequencing of T cells of patient 22,

Fig. 4

shows nucleotide and amino acid sequences of CDR3 β regions of tumour-specific TCR which have been determined by in situ sequencing of T cells of patient 22,

Example 1

Analysis of T cell receptors in HLA-A2 patients with kidney cell carcinoma

Cytotoxic CD8⁺ T cells were identified in kidney cell patient 26 which lysed autologous tumour cells with a HLA-A2 restricted mechanism. The T cells have a high tumour specificity since short-term cultures of normal kidney cells are not recognized. The determinants recognized by the TIL of patient 26 were also found on other tumours of patients which carry the HLA-A2 gene in particular the widespread HLA-A*0201 allele. Normal kidney cells of these patients were not lysed. These results show that the kidney carcinoma cells of patient 26 express a tumour determinant i.e. a tumour-associated peptide/HLA-A2 complex which is also present on the tumours of other patients.

Total RNA is isolated from T cells in order to identify and sequence tumour-specific TCR. For this the cells in suspension are washed with PBS and the cell pellet is resuspended with 0.2 ml RNazol-B per 1×10^6 cells. 2 ml RNazol-B per 100 mg tissue is added to extract the RNA from the tissue. After mechanically resuspending the lysates several times and optionally adding yeast tRNA as a carrier matrix, the RNA is extracted by adding 0.2 ml chloroform per 2 ml homogenate, subsequently mixing for 15 sec. and storing for 5 minutes on ice.

After a centrifugation step at 12,000 g for 15 min at 4°C, the aqueous phase is removed and transferred into a new reaction vessel. The first precipitation of the RNA is carried out by adding an identical volume of

isopropanol and subsequently storing for at least 15 min at 4°C. After centrifuging for 15 min at 12,000 g and 4°C, the RNA is obtained as a white pellet at the bottom of the vessel.

After discarding the supernatant, the RNA pellet is purified of salts by briefly mixing in 75 % ethanol. After centrifuging (7500 g, 4°C, 8 min), the pellet is dissolved in 175 µl water treated with diethylpyrocarbonate (DEPC) and precipitated again with 500 µl ethanol and 75 µl 2 M NaCl for at least 1 h at -20°C. The centrifugation and washing steps after the second precipitation are carried out as described for the first precipitation. After drying the pellets in air, the RNA is resuspended in H₂O-DEPC or 0.5 % SDS, pH 6.5 to 7.0 or 1 mM EDTA, pH 7.0.

Subsequently cDNA is synthesized from the RNA by reverse transcription. For this 3 µg total RNA is incubated for 10 minutes at 55°C with 30 ng P-CαST (a specific primer for the TCRα chain with the sequence 5'-CAC TGA AGA TCC ATC ATC TG-3' shown in SEQ ID NO. 21) and 30 ng P-CβST (a specific primer for the TCRβ chain with the sequence 5'-TAG AGG ATG GTG GCA GAC AG-3' shown in SEQ ID NO. 22) in a reaction volume of 10 µl. Afterwards 38 µl RAV-2-RT buffer (100 mM Tris-HCl pH 8.3; 140 mM KCl; 10 mM MgCl₂; 2 mM dithiothreitol, 0.1 mM of each dNTP), 1 µl (0.75 U) rRNasin and 1 µl (18 U) reverse transcriptase are added by pipette. The reverse transcription is carried out for 1 h at 42°C, followed by a denaturation step at 68°C for 5 min. It is stored until use at -20°C.

Subsequently a polymerase chain reaction is carried out. The primer can be biotinylated in order to enable the

PCR products to be subsequently purified by coupling to a magnetic particulate solid phase (streptavidin-coated beads).

The PCR is carried out using a thermostable DNA polymerase and the following reaction scheme:

95°C 5 min. predenaturation (only at the beginning)

95°C 30 sec DNA denaturation

56°C 1 min annealing

72°C 1 min extension

72°C 10 min filling up all single strands in the reaction solution (only at the end).

The number of reaction cycles in the PCR is usually 30.

The PCR fragments obtained in this manner are sequenced.

When the cytotoxic T cells from patient 26 are cultured and periodically restimulated over a period of 62 and 74 days respectively, a uniform CD8⁺ T cell clone is obtained. The nucleotide and amino acid sequence of the TCR α chain of this T cell clone from patient 26 are shown in SEQ ID NO. 1 and 2. The nucleotide and amino acid sequence of the TCR β chain are shown in SEQ ID NO. 3 and 4.

When the tumour infiltrating lymphocytes from patient 26 were only cultured for 24 days, the T cell clone was not found to be homogeneous but rather a mixture of several T cell species. The CDR α regions of these T cell species contained a total of two further sequences (SEQ ID NO. 5 and 6 and 7 and 8) in addition to the amino acid

sequence shown in SEQ ID NO. 2. In addition to the amino acid sequence shown in SEQ ID NO. 4, the CDR3 β regions contained further closely related sequences (SEQ ID NO. 9 and 10, 11 and 12, 13 and 14, 15 and 16, 17 and 18 and 19 and 20).

Furthermore the T cells of patient 26 were sequenced in situ i.e. sequenced without prior culturing. In this process a series of individual sequences was obtained for the CDR3 α region which are shown in Fig. 1. Circa 60 % of all sequences of the α chain correspond to the sequences previously described. A further 20 % correspond to very similar sequences.

Also in the case of the CDR3 regions of the β chain it was found that a total of 70 % of the examined T cells of patient 26 had a very similar sequence pattern (Fig. 2).

Peripheral blood samples from patient 26 were analyzed for T cell receptors which have features of tumour-specific T cell receptors over 4 years in all. It was found that such sequences only occurred with a frequency of about 1/150,000 T cells.

Cytotoxicity investigations showed that the tumour-specific T cells isolated from patient 26 could also lyse tumour cells of patient 22 which also carry the HLA-A*0201 allele. Tumour infiltrating T cells from patient 22 could in turn lyse tumour cells from patient 26. A sequencing of the T cell receptors from patient 22 yielded the results shown in Fig. 3 for the CDR3 α region and in Fig. 4 for the CDR3 β region.

Example 2

Expression of T cell receptors

2.1 Expression of tumour specific T cell receptors in human or murine T cell lines

The nucleic acid sequences identified in example 1 which code for tumour specific TCR α and β chains are cloned into eukaryotic human and murine expression vectors. The human expression vector is described in Chung et al. (Proc. Natl. Acad. Sci. USA 92 (1995): 3712-3716). The murine vectors are described in Gabert et al. (Cell 50 (1987: 545-554) and Gregoire et al. (Proc. Natl. Acad. Sci. USA 88 (1991): 8077-8081).

The TCR DNA can either be cloned from rearranged genomic DNA or from cDNA. Basically two cloning strategies are available: firstly the isolation of very long TCR α and β DNA fragments from the genome of mature T cells which contain several Kb long 5' flanking sequences with all regulatory elements required for expression. Alternatively vectors can be selected which already contain the natural 5' regulatory elements and in which only short fragments coding for the variable regions have to be cloned in (Kouskoff et al. J. Immunol. Methods 180 (1995): 273-280). In the latter method the sequence of the variable region (including the leader sequence) is examined for mistakes by sequencing after amplification by means of specific PCR and subsequently introduced into the vector after digestion with appropriate restriction

endonucleases.

The PCR α and β chains can either be cloned into a common vector or into two different vectors. Each of the vectors used contains a selection marker which enables the positive selection of successfully transfected cells after transfection of the recipient cells with the recombinant plasmid. Preferred selection markers are for example the gene for neomycin resistance (neo) or the gene for xanthine-guanine-phosphoribosyl-transferase (GPT).

2.2 Expression of functional T cell receptors as single chain constructs

Similarly to antibodies it is possible to express TCR as single chain constructs in eukaryotic cells (Chung et al., Proc. Natl. Acad. Sci. USA 91 (1994): 12654-12658). In this method a construct is prepared which also contains the constant domain of the β chain in addition to the variable domains of the TCR α and β chain. The individual domains are amplified by means of PCR as described in example 1 after isolation of the corresponding RNA and reverse transcription. In this process suitable restriction cleavage sites are inserted at the ends of the amplification products. The individual fragments are then ligated together as follows in a eukaryotic expression vector (e.g. pBJ-Neo) which carries a positive selection marker: the variable TCR α and β domains comprising leader, V-(D)- and J exon are separated by a linker sequence e.g. a DNA fragment coding for the amino acid sequence

(GGGGS)₃. The exon for the constant TCR β domain is ligated directly to the variable β domain.

Alternatively coding sequences for a GPI anchor (Lin et al., Science 249 (1990): 677-679) or for example the transmembrane part and the intracellular domain of the CD3 ζ chain (Engel et al., Science 256 (1992): 1318-1321) can be ligated to the 3' end of this construct. After transfection of these constructs in eukaryotic cells, the former enables the production of soluble TCR molecules which can be used as an immunogen to produce antibodies. The latter enables the functional analysis of the construct in biological systems.

2.3 Production of soluble human TCR fragments in E. coli

Large amounts of soluble TCR fragments can be produced in E. coli as single chain polypeptides (Hilyard et al., Proc. Natl. Acad. Sci. USA 91 (1994): 9057-9061).

For this various genes or gene fragments are cloned into an inducible prokaryotic vector e.g. pUC19. The fragments to be ligated are reamplified by means of specific PCR in the process of which suitable restriction cleavage sites are added.

The following fragments are cloned into the vector in the order shown:

1. A prokaryotic signal sequence e.g. the pelB-leader sequence from the pectate lyase gene of

*Erwinia carolovor*a (Ward et al., Nature 341 (1989): 8646-8650) which causes a secretion of the polypeptide into the periplasm of the host bacterium.

2. The variable PCR α and β chain fragments from a tumour-specific TCR. These fragments are preferably separated by a linker e.g. the linker shown in example 2.2. which improves the solubility and the flexibility of the synthesized molecule.
3. A nucleotide sequence coding for a tail made of several e.g. 6 histidine residues which enables the recombinant polypeptide to be isolated by affinity chromatography e.g. by nickel chelate chromatography.

Example 3

Production of antibodies against tumour-specific T cell receptors

Mice are immunized with the appropriate antigen to produce antisera or monoclonal antibodies against tumour-specific TCR. The immunization is carried out according to the protocols given by Harlow, E. and David, C., Antibodies. A Laboratory Manual, Cold Spring Harbor Laboratory, 1988. TCR expressing cells (example 2.1) or soluble TCR (example 2.2 or example 2.3) can for example be selected as antigens.

Alternatively the soluble TCR used for the immunization

can also be produced as chimeric proteins which are composed of a variable TCR region, a truncated constant TCR region and a constant immunoglobulin region (cf. e.g. Gregoire et al. (1991), Supra). For this the specific variable TCR α and β regions are each cloned into a plasmid which already contains the first exon, a corresponding C region and an IgGk domain. Both plasmids additionally contain a positive selection marker and the regulatory elements required for correct expression. Both plasmids are then used to transfect a mouse myeloma cell line which does not express endogenous heavy and light Ig chains. After the transfection is completed both chimeric chains are synthesized and preferentially secreted as heterodimers.

Alternatively a TCR protein antigen for immunizing mice can be constructed as follows: A human V gene segment is fused to a TCR gene segment composed of (D), J and C gene segments from a mouse T cell hybridoma i.e. the gene segments are cloned in this order into a eukaryotic expression vector (Choi et al., Proc. Natl. Acad. Sci. USA 88 (1991): 8357-8361). The human sequence is obtained from the corresponding cDNA by means of PCR by amplifying the V region. Such constructs are then used to transfect mouse T cell hybridomas which provide all components apart from the corresponding transfected chains. Since the plasmids also code for selection markers, transfectants can be positively selected by an appropriate medium. Since these transfectants represent mouse T cells which express a human V region, mice that are immunized with such cells only produce antibodies against this foreign human sequence.

Example 4

Identification of the peptide ligands of tumour specific T cells

Poly-A⁺ mRNA is isolated from a kidney cell carcinoma line using a commercial kit (Fastrack/Invitrogen) and converted into double-stranded cDNA using the Superscript Choice System kit (Gibco) using a NotI/Oligo-dT primer for the first strand synthesis. The cDNA is ligated with BstXI adaptors and cleaved with NotI. High molecular size fractionated cDNA is selected and cloned into the vector pcDNA1/Amp (Invitrogen) cleaved with BstXI and NotI.

E. coli DH5 α cells are transformed by electroporation with the recombinant plasmids and selected with ampicillin. The cDNA bank obtained in this manner is divided into 1500 pools each comprising approximately 100 clones. Each pool is amplified to saturation and the plasmid DNA is isolated from this by alkaline lysis without phenol extraction.

In each case approximately 100 ng plasmid DNA of a pool is transfected together with 50 ng plasmid DNA of the same vector which carries the HLA-A*0201-cDNA (gene bank, ACC No.: M32322, K02883, M84379, X02457) into 15000 COS7 cells according to the DEAE-dextran-chloroquine method. Alternatively the COS7 cells can also be transfected with the HLA-A*0201 DNA and the stable transfectants obtained in this manner can be used as recipient cells.

24-48 hours after transfection the COS7 cells are tested

for their ability to stimulate the release of TNF by tumour specific cytotoxic T cells (CTL). A test is carried out in each case with 200 pools i.e. 200 independent transfections of COS7 cells.

For this 3000 CTL are added to the wells of microtitre plates containing COS7 transfectants. After 18 hours the supernatant of the medium is collected and its TNF content is determined using an activity test in which TNF sensitive cell lines such as the mouse fibroblast cell lines WEHI 164 or L929 are lysed by TNF. Viable cultures can be distinguished from lysed cells by a colorimetric test using 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT).

A new cycle of COS7 transfection was carried out for each positive microculture in which smaller pools of bacteria from the original pool containing a total of 100 clones were used in each case. This procedure is repeated until a single plasmid is identified which can induce the TNF release from the specific TCL after co-expression with HLA-A*0201 cDNA in COS7 cells.

The sequence of the plasmid insertion is determined by standard methods. The confirmation that this sequence codes for the tumour peptide is achieved by transfecting normal human HLA-A*0201 cells which are not lysed by the tumour specific CTL. These cells are sensitive for a lysis after transfection with the corresponding cDNA. Furthermore the tumour specific expression of the identified cDNA is determined by Northern blot using the cDNA as a probe. This probe is used for hybridization to mRNA from various tumour cell lines of normal tissue samples.

The tumour specific peptide can be identified by various methods. The corresponding protein sequence is derived from the cDNA sequence and screened for binding motifs which had been identified in other HLA-A*0201 binding peptides. Synthetic peptides which overlap with potential HLA-A*0201 binding regions are then tested for their ability to activate CTL after incubation with HLA-A*0201 cells. Alternatively overlapping peptides of 8-9 amino acids in length can be produced by synthesis and tested in a similar manner.

Example 5

Production of transgenic mice

Total RNA is isolated from a specific T cell clone and cDNA is synthesized by reverse transcription (cf. example 1). Using primers specific for the V region, TCR-cDNA for the V α and V β regions is amplified and cloned into TCR gene cassettes which contain constant regions and the necessary regulation elements for expression. Separate cassettes for TCR α and TCR β sequences are known which each carry a different selection marker (Kouskoff et al., (1995), Supra).

Fertilized mouse oocytes are simultaneously microinjected with DNA from the TCR α as well as from the TCR β cassettes. The injected oocytes are transferred back into female mice (Mellor, A.L., Transgenesis and the T cell receptor. in: T cell receptors (1995), J. I. Bell, M. J. Owen and E. Simpson, eds. pp 194-223, Oxford University Press, Oxford, New York, Tokyo).

The introduction of productively rearranged TCR genes in

the mouse has a major influence on the TCR repertoire since rearranged TCR foreign genes prevent the further rearrangement of endogenous TCR genes. Consequently nearly all thymocytes and T cells express the heterologous TCR clonotype so that the TCR repertoire in such mice is essentially monoclonal.

Transgenic mice are identified by genotype analysis using probes which are specific for the DNA contained in the foreign gene that does not occur in the mouse genome. This can either be carried out by Southern blot hybridization or preferably by PCR.

Transgenic descendants of the mice are obtained by crossing with non-transgenic mice of a suitable strain, typing the descendants and using them for further crossing.

Claims

1. Nucleic acid which codes for the α chain of a human T cell receptor, or for a functional derivative or a fragment thereof and which comprises a CDR3 region formed from a combination of a V α 20 and J α 22 gene segment.
2. Nucleic acid which codes for the α chain of a human T cell receptor, or for a functional derivative or a fragment thereof and comprises a CDR3 region selected from:

- (a) a nucleotide sequence coding for the amino acid sequence

Y C L (X₁...X_n) S A R Q L T F (I)

in which X₁ ... X_n represents a sequence of
3-5 amino acids,

- (b) a nucleotide sequence which codes for an amino acid sequence which is at least 80 % identical with the amino acid sequence from (a), or
- (c) a nucleotide sequence which codes for an amino acid sequence with an equivalent recognition specificity for the peptide component of the T cell receptor ligands.

3. Nucleic acid as claimed in claim 2,
w h e r e i n
the amino acid sequence $X_1 \dots X_n$ is selected from
the group comprising the amino acid sequences VGG,
VLSG, ATG, VSG, DSG, VVSG, ALAG, APSG and VGR.
4. Nucleic acid as claimed in claim 3,
w h e r e i n
the amino acid sequence $X_1 \dots X_n$ is selected from
the group comprising amino acid sequences VGG, VLSG
and ATG.
5. Vector,
w h e r e i n
it contains at least one copy of a nucleic acid as
claimed in one of the claims 1 to 4.
6. Cell,
w h e r e i n
it expresses a nucleic acid as claimed in one of
the claims 1 to 4.
7. Cell,
w h e r e i n
it is transformed with a nucleic acid as claimed in
one of the claims 1 to 4 or with a vector as
claimed in claim 5.
8. Polypeptide,
w h e r e i n
it is coded by a nucleic acid as claimed in one of
the claims 1 to 4.

9. Polypeptide as claimed in claim 8,
w h e r e i n
it comprises the variable domain of the α chain of
a human T cell receptor.
10. Nucleic acid which codes for the β chain of a human
T cell receptor, or for a functional derivative or
a fragment thereof and comprises a CDR3 region
formed from a combination of a $V\beta 22$ gene segment, a
 $D\beta 1$ or $D\beta 2$ gene segment and a $J\beta$ gene segment in
particular a $J\beta 2.1$, $J\beta 2.3$ or $J\beta 2.7$ gene segment.
11. Nucleic acid which codes for the β chain of a human
T cell receptor, or for a functional derivative or
a fragment thereof and comprises a CDR3 region
which is selected from:

- (a) a nucleotide sequence coding for the amino acid
sequence

C A (X'₁ ... X'_n) Y/D E Q Y F (II)

in which X'₁ ... X'_n represents a sequence of
5-7 amino acids,

- (b) a nucleotide sequence coding for the amino acid
sequence

C A (X''₁ ... X''_n) N E Q F F (III)

in which X''₁ ... X''_n represents a sequence of
5-7 amino acids,

- (c) a nucleotide sequence coding for the amino acid sequence

C A (X''''₁ ... X''''_n) D T Q Y F (IV)

in which X''''₁ ... X''''_n represents a sequence of 5-7 amino acids,

- (d) a nucleotide sequence which codes for an amino acid sequence that is at least 80 % identical with an amino acid sequence from (a), (b) or/and (c), or

- (e) a nucleotide sequence which codes for an amino acid sequence with an equivalent recognition specificity for the peptide component of the T cell receptor ligand.

12. Nucleic acid as claimed in claim 11,

w h e r e i n

the amino acid sequence X'₁ ... X'_n is selected from the group comprising SSETNS, SSETSS, TSGTAS, RSGTGS, SSGTDS, SSGTRS, SSGSDS, SSSTGS, SSSTVS, SSSTLS, SSSTLF, SSSTAS, SSHTDS, SSDTLS and SRWDSE.

13. Nucleic acid as claimed in claim 12,

w h e r e i n

the amino acid sequence X'₁ ... X'_n represents SSETNS, SSGTDS, TSGTAS or RSGTGS.

14. Nucleic acid as claimed in claim 11,
w h e r e i n
the amino acid sequence $X''_1 \dots X''_n$ represents
SSGTSSY or SSDQGM or the amino acid sequence
 $X'''_1 \dots X'''_n$ represents SADSFK.
15. Vector,
w h e r e i n
it contains at least one copy of a nucleic acid as
claimed in one of the claims 10 to 14.
16. Cell,
w h e r e i n
it expresses a nucleic acid as claimed in one of
the claims 10 to 14.
17. Cell,
w h e r e i n
it is transformed with a nucleic acid as claimed in
one of the claims 10 to 14 or with a vector as
claimed in claim 15.
18. Polypeptide,
w h e r e i n
it codes for a nucleic acid as claimed in one of
the claims 10 to 14.
19. Polypeptide as claimed in claim 18,
w h e r e i n
it comprises the variable domain of the β chain of
a human T cell receptor.

20. Polypeptide,
w h e r e i n
it has T cell receptor properties and is composed
of a polypeptide as claimed in claim 8 or 9 as well
as a polypeptide as claimed in claim 18 or 19 as
subunits.
21. Polypeptide as claimed in one of the claims 8, 9,
18, 19 or 20,
w h e r e i n
it is coupled to a labelling group or a toxin.
22. Polypeptide as claimed in one of the claims 8, 9,
18, 19, 20 or 21,
w h e r e i n
it is present in an oligomerized form.
23. Antibody against a polypeptide as claimed in one of
the claims 8, 9, 18, 19, 20, 21 or 22 which is
directed against a region which is responsible for
recognizing the peptide ligand.
24. Antibody as claimed in claim 23,
w h e r e i n
it is directed towards a CDR3 region.
25. T cell,
w h e r e i n
it contains a T cell receptor as claimed in claim
20.

26. Pharmaceutical composition which contains as active component a nucleic acid as claimed in one of the claims 1 to 4 or 10 to 14, a polypeptide as claimed in one of the claims 8, 9 or 18 to 23, a peptide ligand against the polypeptide, an antibody as claimed in claim 23 or 24 or a cell as claimed in claim 6, 7, 16, 17 or 25 optionally together with other active components as well as common pharmaceutical auxiliary agents, additives or carrier substances.
27. Use of a pharmaceutical composition as claimed in claim 26 for the production of an agent for the diagnosis of tumour diseases or a predisposition for a tumour disease.
28. Use of a pharmaceutical composition as claimed in claim 26 for the production of an agent for monitoring the course of the disease in a tumour disease.
29. Use as claimed in claim 27 or 28,
w h e r e i n
the detection of T cells that express a polypeptide as claimed in claim 20 as the T cell receptor is carried out in a sample liquid by a nucleic acid hybridization assay, an immunoassay, a test for the binding of specific peptide ligands or a specific T cell activity test.
30. Use of a pharmaceutical composition as claimed in claim 26 for the production of an agent for the prevention or therapy of a tumour disease.

31. Use as claimed in claim 30,
w h e r e i n
the agent is suitable for the stimulation of the
growth of T cells that express a polypeptide as
claimed in claim 20 as a T cell receptor.
32. Use as claimed in claim 31,
w h e r e i n
the agent is suitable for growth stimulation of the
T cells in vivo.
33. Use as claimed in claim 31 or 32,
w h e r e i n
the agent for growth stimulation comprises the
peptide ligand of the T cell receptor or/and the
entire molecule from which the peptide ligand is
derived or a fragment thereof.
34. Use as claimed in claim 31 or 32,
w h e r e i n
the growth stimulation includes an antibody that
specifically activates the T cell receptor.
35. Use as claimed in claim 31,
w h e r e i n
the growth stimulation is carried out by isolating
specific T cells, in vitro expansion and subsequent
administration of expanded T cells.
36. Use as claimed in one of the claims 27 to 35,
w h e r e i n
the tumour disease is a kidney cell carcinoma.

37. Process for the isolation of T cells that express a polypeptide as claimed in claim 20 as a T cell receptor,
w h e r e i n
a sample containing T cells is contacted with an agent that binds specifically to the CDR3 region of the T cell receptor, T cells that react with the agent are identified and optionally separated from other T cells.
38. Process as claimed in claim 37,
w h e r e i n
the agent is selected from the peptide ligand of T cells, a MHC peptide complex containing the peptide ligand or/and an anti-TCR antibody.
39. Process as claimed in claim 37 or 38 additionally comprising an in vitro expansion of T cells.
40. Process for the isolation of T cells which express a polypeptide as claimed in claim 20 as the T cell receptor,
w h e r e i n
nucleic acid sequences that code for the T cell receptor are introduced into a T cell line and are made to express therein.
41. Process for the isolation of T cells that express a polypeptide as claimed in claim 20 as the T cell receptor,
w h e r e i n
nucleic acid sequences which code for the T cell

receptor are introduced into the germ line of an animal and the T cells are isolated from the resulting transgenic animal or descendants thereof.

42. Transgenic animal,
w h e r e i n
it expresses a polypeptide as claimed in claim 20
as the T cell receptor.
43. Method for the identification of peptide ligands of
a T cell receptor as claimed in claim 20 comprising
the steps:
 - (a) isolating RNA from tumour tissue,
 - (b) converting the RNA into double-stranded cDNA
molecules,
 - (c) introducing the cDNA molecules into host cells
to obtain a cDNA bank,
 - (d) transfecting eukaryotic recipient cells with
aliquots of the cDNA bank wherein (i)
cotransfection with HLA-A*0201 DNA is carried
out or (ii) HLA-A*0201 positive recipient
cells are used,
 - (e) testing the transfected recipient cells for
their ability to stimulate T cells,
 - (f) identifying a cDNA sequence which codes for
the antigen which contains the peptide ligand
and

(g) identifying the sequence of the peptide ligand.

44. Method as claimed in claim 43,
w h e r e i n
step (e) comprises testing for the ability to lyse
TNF-sensitive cells.

Abstract

The present invention concerns new nucleic acid and amino acid sequences of the human T cell receptor and their use for the diagnosis and therapy of carcinomas in particular of kidney cell carcinomas.

Results #26 tumour i.s. CDR3 α -Region

Fragment	TCRAV20S1	N-Region	TCRAJ
<u>Clones</u> (14/54)	C L V G TGCCTCGTGGG	TG Va20 or Ja22 coded:	G S A R Q L T F GTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (1/54)	C L V G TGCCTCGTGGG	A	G S A R Q L T F GGTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (11/54)	C L V TGCCTCGT	L CCT	S G S A R Q L T F TTCTGGTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (5/54)	C L V TGCCTCGTG	L CT	S G S A R Q L T F TTCTGGTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (2/54)	C L TGCCTCG	A CTA	T G S A R Q L T F CTGGTTCTTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (2/54)	C L TGCCTCG	V T	S G S A R Q L T F TTCTGGTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (2/54)	C L V TGCCTCGTGG	V S TCTCC	G S A R Q L T F GGTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (1/54)	C L TGCCTCG	D S ACTCC	G S A R Q L T F GGTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (1/54)	C L TGCCTCG	D AC	S G S A R Q L T F TCTGGTTCTGCAAGGCAACTGACCTT TCRAJ22
<u>Clones</u> (2/54)	C L TGCCTCG	A L A CCCTGGGG	G S A R Q L T F GGTTCTGCAAGGCAACTGACCTT TCRAJ22

Clones (1/54)C L
TGCCTCGA L A
CCCTGGCGG S A R Q L T F
GGTTCTGCAAGGCAACTGACCTT
TCRAJ22Clones (1/54)C L
TGCCTCGA P
CGCCCS G S A R Q L T F
TCTGGTTCTGCAAGGCAACTGACCTT
TCRAJ22Clones (2/54)C L
TGCCT

TC

P S G S A R Q L T F
CTTCTGGTTCTGCAAGGCAACTGACCTT
TCRAJ22

G G T T C T G C A A G G C A A C T G A C C T T

Results #26 tumour i.s. CDR3 β -Region

Fragment	TCRBV22S1	N-TCRBD-N	TCRBJ
Clones (8/62)	C A S S TGTGCCAGCAG	E T N CGAAACTAA TCRBD2	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A S S TGTGCCAGCAGT	E T N GAAACTAAT TCRBD2	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAGT	E T S GAAACTTCT TCRBD2	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAGT	E T S GAAACAAG TCRBD1	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A TGTGCCA	T S G T A CCTCCGGGACAGCT TCRBD1	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A R TGTGCCAG	S G T G ATCCGGGACAGG TCRBD1	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A S S TGTGCCAGCAGT	G T D GGGACGGA TCRBD1/2	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAGT	G T D GGCACAGA TCRBD1	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAG	G T D CGGGACAGAT TCRBD1	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7

Fig. 2/2

Clone (1/62)	C A S S TGTGCCAGCAGT	G T R <u>GGGACTCGT</u> TCRBD2	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAGT	G T R <u>GGGACACGT</u> TCRBD1	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A S S TGTGCCAGCAGT	G T S S <u>GGAAGTAGCTCTT</u> TCRBD2	Y N E Q F F ACAATGAGCAGTCTT TCRBJ2S1 (TCRBJ2S7 sehr ähnlich)

clone (1/62)	C A S S TGTGCCAGCAGT	G S D <u>GGGTCCGA</u> TCRBD1/2	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7

Clones (5/62)	C A S S TGTGCCAGCAGT	S T G <u>TCGACAGGG</u> TCRBD1	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A S S TGTGCCAGCAG	S T V <u>CTCGACGGT</u> TCRBD1/2	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAGT	S T L <u>TCAACATTA</u> TCRBD2	S Y E Q Y F TCCTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAGT	S T L F <u>TCAACATTATT</u> TCRBD2	Y E Q Y F CTACGAGCAGTACTT TCRBJ2S7
Clone (1/62)	C A S S TGTGCCAGCAGT	S T A <u>TCGACAGC</u> TCRBD1	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A S S TGTGCCAGCAG	H T D <u>CCACACCGA</u> TCRBD1	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7
Clones (2/62)	C A S S TGTGCCAGCAGT	D T L <u>GACACCT</u> TCRBD1	S Y E Q Y F CTCCTACGAGCAGTACTT TCRBJ2S7

Clones (2/34) Y C L A T G S A R Q L T F
TACTGCCTCG CTA CTGGTTCTGCAAGGCAACTGACCTT
TCRAJ22

Results #22 tumour i.s. CDR3 β -Region

Clones (10/28)	C A S TGTGCCAG	A D S F K TGCCGATTCTTTTAA TCRBD2	D T Q Y F AGATACGCAGTATTT TCRBJ2S3
----------------	-------------------	----------------------------------------	------------------------------------------

Clones (4/28)	C A S S TGTGCCAGCAG	E T N CGAAACTAA TCRBD2	S Y E Q Y F CTCCTACGAGCAGTACTT Jb2.7
---------------	------------------------	------------------------------	--------------------------------------------

Clones (1/28)	C A S S TGTGCCAGCAGT	D Q G M GATCAGGGGGATG TCRBD2	N E Q F F AATGAGCAGTTCTT TCRBJ2S1
---------------	-------------------------	------------------------------------	-----------------------------------------

Clones (1/28)	C A S R TGTGCCAGCAG	W D S E GTGGGACTCCGAGG TCRBD2	D E Q Y F ACGAGCAGTACTT TCRBJ2S7
---------------	------------------------	-------------------------------------	----------------------------------------

SEQUENCE PROTOCOL

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Boehringer Mannheim GmbH
(B) ROAD: Sandhofer Str. 112-132
(C) CITY: Mannheim
(E) COUNTRY: Germany
(F) POSTAL CODE: 68305

(ii) TITLE OF INVENTION: T cells specific for kidney carcinoma

(iii) NUMBER OF SEQUENCES: 22

(iv) COMPUTER-READABLE FORM:

(A) DATA CARRIER: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, version # 1.30 (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1341 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) POSITION: 1..801

(ix) FEATURE:

(A) NAME/KEY: sig_peptide
(B) POSITION: 1..54

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) POSITION: 55..801

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATG AGG CAA GTG GCG AGA GTG ATC GTG TTC CTG ACC CTG AGT ACT TTG	48
Met Arg Gln Val Ala Arg Val Ile Val Phe Leu Thr Leu Ser Thr Leu	
-18 -15 -10 -5	
AGC CTT GCT AAG ACC ACC CAG CCC ATC TCC ATG GAC TCA TAT GAA GGA	96
Ser Leu Ala Lys Thr Thr Gln Pro Ile Ser Met Asp Ser Tyr Glu Gly	
1 5 10	
CAA GAA GTG AAC ATA ACC TGT AGC CAC AAC AAC ATT GCT ACA AAT GAT	144
Gln Glu Val Asn Ile Thr Cys Ser His Asn Asn Ile Ala Thr Asn Asp	
15 20 25 30	
TAT ATC ACG TGG TAC CAA CAG TTT CCC AGC CAA GGA CCA CGA TTT ATT	192
Tyr Ile Thr Trp Tyr Gln Gln Phe Pro Ser Gln Gly Pro Arg Phe Ile	
35 40 45	

ATT Ile	CAA Gln	GGA Gly	TAC Tyr 50	AAG Lys	ACA Thr	AAA Lys	GTT Val	ACA Thr 55	AAC Asn	GAA Glu	GTG Val	GCC Ala	TCC Ser 60	CTG Leu	TTT Phe	240	
ATC Ile	CCT Pro	GCC Ala 65	GAC Asp	AGA Arg	AAG Lys	TCC Ser	AGC Ser 70	ACT Thr	CTG Leu	AGC Ser	CTG Leu	CCC Pro 75	CGG Arg	GTT Val	TCC Ser	288	
CTG Leu	AGC Ser 80	GAC Asp	ACT Thr	GCT Ala	GTG Val	TAC Tyr 85	TAC Tyr	TGC Cys	CTC Leu	GTG Val	GGT Gly 90	GGT Gly	TCT Ser	GCA Ala	AGG Arg	336	
CAA Gln 95	CTG Leu	ACC Thr	TTT Phe	GGA Gly	TCT Ser 100	GGG Gly	ACA Thr	CAA Gln	TTG Leu	ACT Thr 105	GTT Val	TTA Leu	CCT Pro	GAT Asp	ATC Ile 110	384	
CAG Gln	AAC Asn	CCT Pro	GAC Asp	CCT Pro 115	GCC Ala	GTG Val	TAC Tyr	CAG Gln	CTG Leu 120	AGA Arg	GAC Asp	TCT Ser	AAA Lys	TCC Ser 125	AGT Ser	432	
GAC Asp	AAG Lys	TCT Ser	GTC Val 130	TGC Cys	CTA Leu	TTC Phe	ACC Thr	GAT Asp 135	TTT Phe	GAT Asp	TCT Ser	CAA Gln	ACA Thr 140	AAT Asn	GTG Val	480	
TCA Ser	CAA Gln	AGT Ser 145	AAG Lys	GAT Asp	TCT Ser	GAT Asp	GTG Val 150	TAT Tyr	ATC Ile	ACA Thr	GAC Asp	AAA Lys 155	ACT Thr	GTG Val	CTA Leu	528	
GAC Asp	ATG Met 160	AGG Arg	TCT Ser	ATG Met	GAC Asp	TTC Phe 165	AAG Lys	AGC Ser	AAC Asn	AGT Ser	GCT Ala 170	GTG Val	GCC Ala	TGG Trp	AGC Ser	576	
AAC Asn 175	AAA Lys	TCT Ser	GAC Asp	TTT Phe	GCA Ala 180	TGT Cys	GCA Ala	AAC Asn	GCC Ala	TTC Phe 185	AAC Asn	AAC Asn	AGC Ser	ATT Ile	ATT Ile 190	624	
CCA Pro	GAA Glu	GAC Asp	ACC Thr	TTC Phe 195	TTC Phe	CCC Pro	AGC Ser	CCA Pro	GAA Glu 200	AGT Ser	TCC Ser	TGT Cys	GAT Asp 205	GTC Val	AAG Lys	672	
CTG Leu	GTC Val	GAG Glu	AAA Lys 210	AGC Ser	TTT Phe	GAA Glu	ACA Thr	GAT Asp 215	ACG Thr	AAC Asn	CTA Leu	AAC Asn	TTT Phe 220	CAA Gln	AAC Asn	720	
CTG Leu	TCA Ser	GTG Val 225	ATT Ile	GGG Gly	TTC Phe	CGA Arg	ATC Ile 230	CTC Leu	CTC Leu	CTG Leu	AAA Lys	GTG Val 235	GCC Ala	GGG Gly	TTT Phe	768	
AAT Asn 240	CTG Leu	CTC Leu	ATG Met	ACG Thr	CTG Leu	CGG Arg 245	CTG Leu	TGG Trp	TCC Ser	AGC Ser	TGAGATCTGC			AAGATTGTAA		821	
GACAGCCTGT			GCTCCCTCGC			TCCTTCCTCT			GCATTGCCCC			TCTTCTCCCT			CTCCAAACAG		881
AGGGAACCTCT			CCTACCCCCA			AGGAGGTGAA			AGCTGCTACC			ACCTCTGTGC			CCCCCGGCA		941
ATGCCACCAA			CTGGATCCTA			CCCGAATTTA			TGATTAAGAT			TGCTGAAGAG			CTGCCAAACA		1001
CTGCTGCCAC			CCCCTCTGTT			CCCTTATTGC			TGCTTGTCAC			TGCCTGACAT			TCACGGCAGA		1061
GGCAAGGCTG			CTGCAGCCTC			CCCTGGCTGT			GCACATTCCC			TCCTGCTCCC			CAGAGACTGC		1121
CTCCGCCATC			CCACAGATGA			TGGATCTTCA			GTGGGTTCTC			TTGGGCTCTA			GGTCCTGGAG		1181

AATGTTGTGA GGGGTTTATT TTTTTTTAAT AGTGTTTCATA AAGAAATACA TAGTATTCTT	1241
CTTCTCAAGA CGTGGGGGGA AATTATCTCA TTATCGAGGC CCTGCTATGC TGTGTGTCTG	1301
GGCGTGTGTGT ATGTCCTGCT GCCGATGCCT TCATTAAAAT	1341

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 267 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met	Arg	Gln	Val	Ala	Arg	Val	Ile	Val	Phe	Leu	Thr	Leu	Ser	Thr	Leu	-18	-15	-10	-5
Ser	Leu	Ala	Lys	Thr	Thr	Gln	Pro	Ile	Ser	Met	Asp	Ser	Tyr	Glu	Gly	1	5	10	
Gln	Glu	Val	Asn	Ile	Thr	Cys	Ser	His	Asn	Asn	Ile	Ala	Thr	Asn	Asp	15	20	25	30
Tyr	Ile	Thr	Trp	Tyr	Gln	Gln	Phe	Pro	Ser	Gln	Gly	Pro	Arg	Phe	Ile	35	40	45	
Ile	Gln	Gly	Tyr	Lys	Thr	Lys	Val	Thr	Asn	Glu	Val	Ala	Ser	Leu	Phe	50	55	60	
Ile	Pro	Ala	Asp	Arg	Lys	Ser	Ser	Thr	Leu	Ser	Leu	Pro	Arg	Val	Ser	65	70	75	
Leu	Ser	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Leu	Val	Gly	Gly	Ser	Ala	Arg	80	85	90	
Gln	Leu	Thr	Phe	Gly	Ser	Gly	Thr	Gln	Leu	Thr	Val	Leu	Pro	Asp	Ile	95	100	105	110
Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser	Lys	Ser	Ser	115	120	125	
Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln	Thr	Asn	Val	130	135	140	
Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp	Lys	Thr	Val	Leu	145	150	155	
Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala	Val	Ala	Trp	Ser	160	165	170	
Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn	Asn	Ser	Ile	Ile	175	180	185	190
Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser	Cys	Asp	Val	Lys	195	200	205	
Leu	Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu	Asn	Phe	Gln	Asn	210	215	220	
Leu	Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys	Val	Ala	Gly	Phe	225	230	235	

Asn Leu Leu Met Thr Leu Arg Leu Trp Ser Ser
 240 245

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 936 base pairs
 (B) TYPE: nucleotide
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) POSITION: 1..933

(ix) FEATURE:
 (A) NAME/KEY: sig_peptide
 (B) POSITION: 1..63

(ix) FEATURE:
 (A) NAME/KEY: mat_peptide
 (B) POSITION: 64..933

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATG GAT ACC TGG CTC GTA TGC TGG GCA ATT TTT AGT CTC TTG AAA GCA	48
Met Asp Thr Trp Leu Val Cys Trp Ala Ile Phe Ser Leu Leu Lys Ala	
-21 -20 -15 -10	
GGA CTC ACA GAA CCT GAA GTC ACC CAG ACT CCC AGC CAT CAG GTC ACA	96
Gly Leu Thr Glu Pro Glu Val Thr Gln Thr Pro Ser His Gln Val Thr	
-5 1 5 10	
CAG ATG GGA CAG GAA GTG ATC TTG CGC TGT GTC CCC ATC TCT AAT CAC	144
Gln Met Gly Gln Glu Val Ile Leu Arg Cys Val Pro Ile Ser Asn His	
15 20 25	
TTA TAC TTC TAT TGG TAC AGA CAA ATC TTG GGG CAG AAA GTC GAG TTT	192
Leu Tyr Phe Tyr Trp Tyr Arg Gln Ile Leu Gly Gln Lys Val Glu Phe	
30 35 40	
CTG GTT TCC TTT TAT AAT AAT GAA ATC TCA GAG AAG TCT GAA ATA TTC	240
Leu Val Ser Phe Tyr Asn Asn Glu Ile Ser Glu Lys Ser Glu Ile Phe	
45 50 55	
GAT GAT CAA TTC TCA GTT GAA AGG CCT GAT GGA TCA AAT TTC ACT CTG	288
Asp Asp Gln Phe Ser Val Glu Arg Pro Asp Gly Ser Asn Phe Thr Leu	
60 65 70 75	
AAG ATC CGG TCC ACA AAG CTG GAG GAC TCA GCC ATG TAC TTC TGT GCC	336
Lys Ile Arg Ser Thr Lys Leu Glu Asp Ser Ala Met Tyr Phe Cys Ala	
80 85 90	
AGC AGC GAA ACT AAC TCC TAC GAG CAG TAC TTC GGG CCG GGC ACC AGG	384
Ser Ser Glu Thr Asn Ser Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg	
95 100 105	
CTC ACG GTC ACA GAG GAC CTG AAA AAC GTG TTC CCA CCC GAG GTC GCT	432
Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala	
110 115 120	

GTG	TTT	GAG	CCA	TCA	GAA	GCA	GAG	ATC	TCC	CAC	ACC	CAA	AAG	GCC	ACA	480
Val	Phe	Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr	
	125					130					135					
CTG	GTG	TGC	CTG	GCC	ACA	GGC	TTC	TAC	CCC	GAC	CAC	GTG	GAG	CTG	AGC	528
Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	Ser	
	140				145					150					155	
TGG	TGG	GTG	AAT	GGG	AAG	GAG	GTG	CAC	AGT	GGG	GTC	AGC	ACA	GAC	CCG	576
Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Ser	Thr	Asp	Pro	
				160					165					170		
CAG	CCC	CTC	AAG	GAG	CAG	CCC	GCC	CTC	AAT	GAC	TCC	AGA	TAC	TGC	CTG	624
Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	Leu	
			175					180					185			
AGC	AGC	CGC	CTG	AGG	GTC	TCG	GCC	ACC	TTC	TGG	CAG	AAC	CCC	CGC	AAC	672
Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	Asn	
		190					195					200				
CAC	TTC	CGC	TGT	CAA	GTC	CAG	TTC	TAC	GGG	CTC	TCG	GAG	AAT	GAC	GAG	720
His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu	
	205					210					215					
TGG	ACC	CAG	GAT	AGG	GCC	AAA	CCT	GTC	ACC	CAG	ATC	GTC	AGC	GCC	GAG	768
Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu	
	220				225					230					235	
GCC	TGG	GGT	AGA	GCA	GAC	TGT	GGC	TTC	ACC	TCC	GAG	TCT	TAC	CAG	CAA	816
Ala	Trp	Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln	Gln	
				240				245						250		
GGG	GTC	CTG	TCT	GCC	ACC	ATC	CTC	TAT	GAG	ATC	TTG	CTA	GGG	AAG	GCC	864
Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	Ala	
			255				260						265			
ACC	TTG	TAT	GCC	GTG	CTG	GTC	AGT	GCC	CTC	GTG	CTG	ATG	GCC	ATG	GTC	912
Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	Val	
		270				275						280				
AAG	AGA	AAG	GAT	TCC	AGA	GGC	TAG									936
Lys	Arg	Lys	Asp	Ser	Arg	Gly										
	285					290										

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 311 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met	Asp	Thr	Trp	Leu	Val	Cys	Trp	Ala	Ile	Phe	Ser	Leu	Leu	Lys	Ala	
-21	-20					-15					-10					
Gly	Leu	Thr	Glu	Pro	Glu	Val	Thr	Gln	Thr	Pro	Ser	His	Gln	Val	Thr	
-5					1				5					10		
Gln	Met	Gly	Gln	Glu	Val	Ile	Leu	Arg	Cys	Val	Pro	Ile	Ser	Asn	His	
			15					20					25			

Leu Tyr Phe Tyr Trp Tyr Arg Gln Ile Leu Gly Gln Lys Val Glu Phe
 30 35 40
 Leu Val Ser Phe Tyr Asn Asn Glu Ile Ser Glu Lys Ser Glu Ile Phe
 45 50 55
 Asp Asp Gln Phe Ser Val Glu Arg Pro Asp Gly Ser Asn Phe Thr Leu
 60 65 70 75
 Lys Ile Arg Ser Thr Lys Leu Glu Asp Ser Ala Met Tyr Phe Cys Ala
 80 85 90
 Ser Ser Glu Thr Asn Ser Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg
 95 100 105
 Leu Thr Val Thr Glu Asp Leu Lys Asn Val Phe Pro Pro Glu Val Ala
 110 115 120
 Val Phe Glu Pro Ser Glu Ala Glu Ile Ser His Thr Gln Lys Ala Thr
 125 130 135
 Leu Val Cys Leu Ala Thr Gly Phe Tyr Pro Asp His Val Glu Leu Ser
 140 145 150 155
 Trp Trp Val Asn Gly Lys Glu Val His Ser Gly Val Ser Thr Asp Pro
 160 165 170
 Gln Pro Leu Lys Glu Gln Pro Ala Leu Asn Asp Ser Arg Tyr Cys Leu
 175 180 185
 Ser Ser Arg Leu Arg Val Ser Ala Thr Phe Trp Gln Asn Pro Arg Asn
 190 195 200
 His Phe Arg Cys Gln Val Gln Phe Tyr Gly Leu Ser Glu Asn Asp Glu
 205 210 215
 Trp Thr Gln Asp Arg Ala Lys Pro Val Thr Gln Ile Val Ser Ala Glu
 220 225 230 235
 Ala Trp Gly Arg Ala Asp Cys Gly Phe Thr Ser Glu Ser Tyr Gln Gln
 240 245 250
 Gly Val Leu Ser Ala Thr Ile Leu Tyr Glu Ile Leu Leu Gly Lys Ala
 255 260 265
 Thr Leu Tyr Ala Val Leu Val Ser Ala Leu Val Leu Met Ala Met Val
 270 275 280
 Lys Arg Lys Asp Ser Arg Gly
 285 290

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS

(B) POSITION: 1..39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGC CTC GTC CTT TCT GGT TCT GCA AGG CAA CTG ACC TTT
Cys Leu Val Leu Ser Gly Ser Ala Arg Gln Leu Thr Phe
295 300

39

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Cys Leu Val Leu Ser Gly Ser Ala Arg Gln Leu Thr Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) POSITION: 1..36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

TGC CTC GCT ACT GGT TCT GCA AGG CAA CTG ACC TTT
Cys Leu Ala Thr Gly Ser Ala Arg Gln Leu Thr Phe
15 20 25

36

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Cys Leu Ala Thr Gly Ser Ala Arg Gln Leu Thr Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) POSITION: 1..39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TGT GCC AGC AGT GGA ACA GAT TCC TAC GAG CAG TAC TTC
Cys Ala Ser Ser Gly Thr Asp Ser Tyr Glu Gln Tyr Phe
15 20 25

39

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Cys Ala Ser Ser Gly Thr Asp Ser Tyr Glu Gln Tyr Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs

(B) TYPE: nucleotide

(C) STRANDEDNESS: both

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) POSITION: 1..39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TGT GCC AGC AGT GAA ACA GAT TCC TAC GAG CAG TAC TTC
Cys Ala Ser Ser Glu Thr Asp Ser Tyr Glu Gln Tyr Phe
15 20 25

39

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Cys Ala Ser Ser Glu Thr Asp Ser Tyr Glu Gln Tyr Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) POSITION: 1..39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TGT GCC AGC AGT GGA ACA GCT TCC TAC GAG CAG TAC TTC
Cys Ala Ser Ser Gly Thr Ala Ser Tyr Glu Gln Tyr Phe
15 20 25

39

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Cys Ala Ser Ser Gly Thr Ala Ser Tyr Glu Gln Tyr Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) POSITION: 1..39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

TGT GCC AGC AGT GGT ACA AAC TCC TAC GAG CAG TAC TTT
Cys Ala Ser Ser Gly Thr Asn Ser Tyr Glu Gln Tyr Phe
15 20 25

39

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

- (ii) TYPE OF MOLECULE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Cys Ala Ser Ser Gly Thr Asn Ser Tyr Glu Gln Tyr Phe
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 39 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 1..39

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

TGT GCC ACC TCC GGG ACA GCT TCC TAC GAG CAG TAC TTC
 Cys Ala Thr Ser Gly Thr Ala Ser Tyr Glu Gln Tyr Phe
 15 20 25

39

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) TYPE OF MOLECULE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Cys Ala Thr Ser Gly Thr Ala Ser Tyr Glu Gln Tyr Phe
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 39 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) POSITION: 1..39

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

TGT GCC AGA TCC GGG ACA GGC TCC TAC GAG CAG TAC TTC
 Cys Ala Arg Ser Gly Thr Gly Ser Tyr Glu Gln Tyr Phe
 15 20 25

39

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 13 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) TYPE OF MOLECULE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:..

Cys Ala Arg Ser Gly Thr Gly Ser Tyr Glu Gln Tyr Phe
 1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleotide
 (C) STRANDEDNESS: single strand
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CACTGAAGAT CCATCATCTG

20

(2) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleotide
 (C) STRANDEDNESS: single strand
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

TAGAGGATGG TGGCAGACAG

20

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

(Insert Title) T cells specific for kidney carcinoma

the specification of which

- (Check one of blocks 1, 2, or 3. See note A on back of this page)
1. ☒ is attached hereto.
2. ☐ was filed on _____ as International PCT Application Serial No. _____ and was amended on _____ (if applicable).
3. ☐ was filed on _____ as U.S. Application Serial No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application for which priority is claimed:

	<u>196 25 191.5</u>	<u>DE</u>	<u>6 June 1996</u>	Priority Claimed
	(Number)	(Country)	(Day/Month/Year Filed)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
(List prior foreign applications. See note B on back of this page)	_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
	_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
	_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No
	_____	_____	_____	<input type="checkbox"/> Yes <input type="checkbox"/> No

(See Note C on back of this page)

☐ See attached list for additional prior foreign applications

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(List Prior U.S. Applications)	(Application Serial Number)	(Filing Date)	(Status) (patented, pending, abandoned)
_____	_____	_____	_____
_____	_____	_____	_____

And I hereby appoint as principal attorneys David T. Nikaido, Reg. No. 22,663; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Robert B. Murray, Reg. No. 22,980; Martin S. Postman, Reg. No. 18,570; E. Marcie Emas, Reg. No. 32,131; Michael G. Gilman, Reg. No. 19,114; Douglas H. Goldhush, Reg. No. 33,125; Juan Carlos Marquez, Reg. No. 34,072; Robert L. Waddle, Reg. No. 35,795; Kevin C. Brown, Reg. No. 32,402; Monica F. Chin, Reg. No. 36,105.

Please direct all communications to the following address: NIKAIDO, MARMELSTEIN, MURRAY & ORAM
Metropolitan Square
655 Fifteenth Street, N.W., Suite 330 - G Street Lobby
Washington, D.C. 20005-5701
(202) 638-5000 Fax: (202) 638-4810

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note D on back of this page) Full name of sole or first inventor Dolores J. SCHENDEL

Inventor's signature *Dolores J. Schendel* 09.06.97 Date

Residence Hans-Sachs-Str. 127, D-80469 München, DE

Citizenship American

Post Office Address same as above